REMARKS

Claims 245-255, 258 and 262 are pending in the above-referenced application. As will be discussed in further detail below, claims 245, 255 and claim 262 have been amended to more distinctly recite the subject matter which Applicants regard as their invention.

Claims 263-269 have been added to recite specific embodiments. Claims 263 and 264 depend from claim 245; claim 263 recites that the incompatible cell is a prokaryotic cell and the compatible cell is a eukaryotic cell; claim 264 recites that the non-eukaryotic polymerase is a prokaryotic viral polymerase which is expressed selectively in a eukaryotic cell. Claim 268 is directed to a method for expressing said polymerase in a eukaryotic cell. Claims 265 and 266 and depend from claim 255 and are also directed to specific embodiments; claim 265 recites that the gene product is selected from the group consisting of sense DNA, sense RNA, antisense RNA, antisense DNA and a combination of the foregoing; claim 266 recites that the incompatible cell is a prokaryotic cell. Claim 267 depends from claim 262 and recites that the incompatible cell is a prokaryotic cell and the compatible cell is a eukaryotic cell. Claim 269 is directed to a method of expressing a gene product using the nucleic acid construct of claim 262. New claims 263-269 are supported by the specification. No new matter has been added.

1. The Rejection Under 35 USC §112, First Paragraph (Written Description I)

Claims 245-254 have been rejected under 35 USC §112, first paragraph as failing to comply with the written description requirement. It is asserted that there is inadequate support for the term "non-eukaryotic polymerase". It is specifically stated:

> To be clear, the instant rejection is based on the supposition that there is inadequate support for the term "non-eukaryotic polymerase". In response to this rejection, applicants have asserted generally that support can be found on page 81 and page 87, However this is not convincing, since the support in the indicated location provides only a few examples which are non-eukaryotic, but are not considered to teach the genus of non-eukaryotic. While it is agreed that applicants have provided examples of non-eukaryotic

> polymerases, such examples are not considered to support the concept that eukaryotic polymerases were ever intended to be excluded, or in contrast, that archaeobacteria polymerases were to be included. A few examples from very broad genus of noneukaryotic polymerases are not considered to provide support for the entire genus of non-eukaryotic polymerases, and the rejection is maintained therefore.

Applicants respectfully traverse the rejection. It is Applicants' position that "non-eukaryotic polymerases" are adequately described. MPEP 2163 II.A.3 states that

> The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice..., reduction drawings... **or** by disclosure of relevant identifying characteristics, i.e., structure of other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus...

It is Applicants position that adequate support was provided in view of the drawings provided (see, for example Figures 27-31 and 47). The stick figures presented in the figures were certainly standard for figures presented by those of ordinary skill in the art to describe the construct of the invention.

Relevant identifying characteristics of the claimed constructs are certainly provided in the specification. For example, pages 88 and 89 describe the claimed constructs. In particular, there is a description of the insertion of an intron sequence within the coding sequence of the polymerase and description of expression in compatible but not in incompatible cells. Further information is provided in Example 19 (pages 145-155).

Applicants further take issue with the assertion that

> While it is agreed that applicants have provided examples of non-eukaryotic polymerases, such examples are not considered to support the concept that eukaryotic polymerases were ever intended to be excluded....

Applicants in response, state that it is not necessary to provide evidence of intent of exclusion of eukaryotic polymerases. It is Applicants' position that by reciting the term "non-eukaryotic polymerases" support needs only to be provided for this term. In Applicants view an adequate number of examples are indeed provided. Three examples are provided on page 87, T3, T7 and SP6.

Applicants note that new claim 263 recites that the noncompatible cell is a prokaryotic cell and the compatible cell is a eukaryotic cell; new claim 264 is directed to a prokaryotic viral polymerase. It is Applicants' position that an adequate description is provided for both of these claims.

In view of the above arguments, Applicants assert that the rejections under 35 USC §112, first paragraph (written description) have been overcome. Therefore, Applicants respectfully request that the rejection be withdrawn.

2. The Rejection Under 35 USC §112, Second Paragraph

Claims 245-255, 258, and 262 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regards as the invention. It is asserted that the phrase "wherein said polymerase is (a) incapable of expression" is considered to be vague and indefinite, "because while nucleic acids encoding polymerases may be capable of expression, polymerases themselves are not capable of expression".

In response, claim 245 has been amended to recite that the polymerase is incapble of being expressed in an incompatible cell due to the presence of the non-native intron. Amended claim 245 is supported by the specification on page 89, lines 14-23. Claims 246-254 depend from claim 245. Therefore, arguments made with respect to claim 245 would apply to these claims as well.

Claims 255, 258 and 262 do not recite a construct comprising a sequence encoding a non-eukaryotic polymerase. Therefore, this rejection would not apply to claims 255, 258 and 262.

In view of the amendment of claim 245 and the above arguments, Applicants assert that the rejection under 35 USC §112, second paragraph has been overcome. Therefore, Applicants respectfully request that the rejection be withdrawn.

3. The Rejection Under 35 USC §112, First Paragraph (Written Description II)

Claims 245-255, 258 and 262 have been rejected under 35 USC §112, first paragraph as filing to comply with the written description requirement. The Office Action specifically states:

> ....The specification does not provide support for the use of any intron, in any polymerase or any bacteriophage polymerase, or any conditionally toxic gene, in any incompatible cell because the specification provides only minimal prophetic description and no exemplification, of any particular intron, polymerase (including bacteriophage polymerase), or toxic gene, or cells compatible or incompatible for whom known structures exist that could be utilized having the claimed function.
>
> As stated above, the specification provides only minimal prophetic description and no exemplification, of any particular intron, polymerase (including bacteriophage polymerase), or toxic gene, or cells compatible or incompatible for whom known structures exist that could be utilized having the claimed function. The specification provides for the use of T3, T7 or SP6 polymerases, and also for the use of certain "consensus" splice donor and acceptor sites for inserting introns. Applicants prophetically suggest that intron "insertion at any of these sites in a gene coding region should not affect subsequent removal of the processing element in a compatible cell." (page 84 of the instant specification).
>
> However, there is significant unpredictability in such intron removal, since such a process requires a complex interaction between the nucleic acid construct and the already existent cellular machinery. A review article by Balvay et al. indicates that the splicing machinery is highly dependent upon recognizing and interacting with such secondary structures in making the splice. Balvay et al. indicates that the addition of a secondary structure to an existing mRNA can cause the cell to splice at a point not normally spliced at, while removal of such a structure can cause splicing to be eliminated (for example see pages 165 bridging to 166). Furthermore, Balvay indicates that the exon plays a significant role in splice site recognition by the cellular splicing machinery. Since one of skill would understand that the nucleotides in the exon remain in the mRNA (or ribozyme) after splicing, applicants claimed

> nucleic acid constructs, *following splicing*, would likely therefore contain elements of these exon recognition sites. Such unpredictability indicates that the genus of nucleic acid constructs comprising any intron in any polymerase (or any bacteriophage polymerase), or any toxic gene, and that are active or inactive depending on whether they are found in cells that are compatible or incompatible is very large.
>
> The fact that the specification discloses only prophetic examples and a few species of polymerases and donor/acceptor splice sites is not considered to constitute a sufficient representative sample of the genus of such constructs. The claims are rejected therefore.

Applicants respectfully traverse the rejection. Applicants, in the specification, actually do provide guidance as to properties of the intron that would lead to inactivation such as a) having the presence of the intron throw the coding sequence out of frame and/or b) using an intron sequence that has at least one stop codon in frame with the target gene sequence or preferably like the SV40 intron, having a stop codons in all three reading frames (see paragraph bridging pages 85 and 86).

The use of this system would be a general strategy that should work for most proteins regardless of what particular function the protein normally carried out, i.e the means disclosed for conditionally inactivating a protein should be universally applicable to most protein targets. Having a stop codon in all three reading frames should be enough to inactivate <u>any protein</u> if the intron is close to the 5' end. Additionally, having a shift in a reading frame should also create the same effect.

The limitation to a polymerase or to an otherwise deleterious enzymatic activity is only in regard to the utility or application of insertion of an intron into a nucleic acid sequence encoding for example the polymerase that otherwise lacks it. In this context, it is only a question of a desirable target that would benefit by a conditional inactivation

In terms of compatible and non-compatible, the skilled user would know which situations this would apply to. Specifically, Example 19 discloses a protein that would normally be expressed in a prokaryotic cell being inactivated by the presence of a control element that would only allow expression in a eukaryotic cell. Other compatible/incompatible situations can be foreseen by the a person skilled in the art. For example, due to evolutionary distance, an intron that is excised from a gene in a

mammalian cell does not have the appropriate sequences to be excised when it is in a yeast cell and vice versa.

Applicants do note that to support the assertions of unpredictability, there is a heavy reliance on Balvay et al. in the Office Action. It is asserted that secondary structures of the target mRNA can create situations where the splice sites are not as expected. Applicants, in response, note that the purpose of the Balvay et al. paper was to counter the prevailing attitude that it is essentially the intron and only the intron sequences that dictate splicing reactions. The examples that Balvay bring up are more in the nature of exceptions to the general rule and attempt to show that the exon sequences around an intron can be responsible for seemingly aberrant results. There are normal rules of how and where splicing will occur and these are predictable and usually take place without a problem. As such the "unpredictability" cited by the Examiner is simply a fact that there are no guarantees that splicing events will always take place as expected but there should be no undue experimentation in following the teachings of the present invention and creating appropriate constructs.

For example, Figure 2 of Balvay et al. shows changing positions between a branch site and a 3' splice site; however, their description says "One of the peculiarities of this reaction...." and "These distances can be considered unusually long...." In short, Balvay et al was discussing an exceptional situation where secondary considerations were used to explain why this reaction did not follow normal rules.

Furthermore, Figure 3 of Balvay, shows a secondary structure which allowed skipping a potential 3' splice site. However, the reference is again discussing circumstances that are infrequently encountered. The discussion of the events shown in Figure 3 is prefaced by "Deshler and his colleagues described a rather unusual situation in the actin gene.....". In a conclusion section they say "RNA folding....explains some exceptions to the scanning mechanism which is supposed to control the choice of 3' splice sites". Again secondary structure formation is used in Balvay as an explanation why a normally predictable reaction did not take place.

It should be noted that the subjects of both Figures 2 and 3 are a discussion of effects dictated by sequences within the intron and not of the flanking sequences of the exon. In Applicants view, it would be evident to one of skill in the art that the choice of a

particular intron would be based upon knowledge of its properties in its native milieu and it would be known if such an intron was following normal rules or had some exceptions.

Applicants respectfully point out that Balvay et al. in a conclusory statement point out that "Although some of these suggestions remain to be proven, it seems rather likely that some exon sequences are involved in the formation of secondary structures which could interact with trans acting factors" (see page 167). Rather than a statement that would imply juxtaposition of exon and exon sequences creates a situation where locations of splicing event are unpredictable, they are only saying that sometimes it's not where it would normally be expected to take place.

Applicants further note that proteins from mRNA's that lack introns are often expressed at lower levels than those from mRNA's having one or more exons. As such, a search of eukaryotic cloning expression vectors shows that many of these have been designed to contain an intron adjacent to a cloning site. Although the donor and acceptor sites are in a non-coding region, it is obvious that if the problems cited by the examiner commonly took place, the subsequent problems in the coding sequence of the protein would render these vectors useless.

In conclusion, Applicants assert that an adequate written description is provided for the claimed subject matter. The specification does provide an adequate teaching of introns, polymerases, toxic genes, compatible and incompatible cells that may be used in the constructs and methods of the present invention. Further, the Balvay et al. reference does not provide evidence of the unpredictability of splicing but merely provides evidence that secondary structures may be involved and describes exceptional situations that may be encountered. Applicants further assert that an adequate written description is provided for new claims 263-269.

In view of the above arguments, Applicants assert that the rejections under 35 USC §112, first paragraph have been overcome. Therefore, Applicants respectfully request that the rejections be withdrawn.

4. The Rejections Under 35 USC §112, First Paragraph-Enablement

Claims 245-255, 258 and 262 have been rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement. The Office Action

specifically states

> Applicants specification does not provide sufficient guidance or examples that would enable a skilled artisan to make the disclosed nucleic acid constructs containing sequences that are spliced out by cellular machinery. ...
>
> The instant invention is wholly dependent upon the ability of the splicing machinery of the cell to interact with such secondary structures in splicing out the intervening sequence to activate the native polymerase or other activity. A review article by Balvay et al. indicates that the splicing machinery is highly dependent upon recognizing and interacting with such secondary structures in making the splice. Balvay et al. indicates that the addition of a secondary structure to an existing mRNA can cause the cell to splice at a point not normally spliced at, while removal of such a structure can cause splicing to be eliminated (for example see pages 165 bridging to 166). Furthermore, Balvay indicates that the exon plays a significant role in splice site recognition by the cellular splicing machinery. Since one of skill would understand that the nucleotides in the exon remain in the RNA after splicing, applicants claimed constructs, which embrace a very broad genus of all polymerases or bacteriophage polymerases, or any toxic protein, *following splicing,* would therefore likely contain elements of these exon recognition sites. Since applicant has not shown that their intervening sequence is spliced out cleanly, or that such disrupted constructs can ever be activated in the cell, and because the prior art indicates uncertainty with respect to the proper splicing of applicants disrupted nucleic acids; one of skill could not depend upon the teachings of the specification or the prior art for overcoming these problems.
>
> Therefore, as indicated above, because secondary structures of RNA vary unpredictably with sequence, and because such secondary structures have a pronounced effect on RNA splicing, and finally because the replacement of even a few nucleotides on a mRNA can abolish all activity of the translated protein, it is maintained that neither the specification nor the prior art arms one of skill with the information necessary to engineer sequences into nucleic acid constructs that will be reliably spliced out to result in a protein with native activity restored.
>
> In order to practice the invention using the specification and the state of the prior art as outlined above, the quantity of experimentation required to practice the invention as claimed

> would therefore require the *de* novo determination of intervening sequences that can be fully spliced out without leaving behind any nucleotides that might interfere with native activity. In the absence of any real guidance from the specification, the amount of experimentation would be undue, and one would have been unable to practice the invention over the scope claimed.

Applicants respectfully traverse the rejection. As noted above, the Balvay et al. reference does not provide evidence of the unpredictability of splicing but merely provides evidence that secondary structures may be involved and describes exceptional situations that may be encountered.

Further, Applicants take issue with the assertion made in the Office Action regarding the unpredictability of splicing. Applicants note that reference was made in the specification concerning work done by Mayeda et al. 1990 where a restriction enzyme fragment of the β-globin gene that contained an intron was inserted into various positions of the β-globin transcript. Very little effect upon splicing efficiency or splicing specificity were observed in this paper since these effects are largely dictated by the intron sequences. As mentioned in the specification, these methods used restriction enzyme fragments and as such the flanking exon sequences immediately adjacent to the splice donor and acceptor sites were always carried along with the intron fragment, but nevertheless each insertion brought the intron into proximity with new sequences and none of the putative problems raised by the Examiner were seen. Normal splicing events took place without any problems with unforeseen (unpredictable) splicing events taking place.

Applicants further note that a Google search of the term "artificial intron" came up with 666 hits and an examination of these documents showed numerous instances where introns were introduced into new environments without any problems. In some cases these "artificial intron" refers to introns that were created de novo and still function; in other cases they are introns derived from nature and their "artificiality" stems form their being put into new environments. A copy of a few of these papers are attached to this response. An example of one article published before the priority date of the above-referenced application is attached hereto as Exhibit 1 is Datta et al., 1996, Mol. Cell Biol. 16:1085-1093.

Applicants position is further supported by Lewin, 1994, Genes V, Oxford University Press, pages 914-915, attached hereto as Exhibit 2 (hereinafter "Lewin"), published one year after Balvay et al. and before the priority date of the instant application. Lewin in the last paragraph of page 914 states:

> Experiments to construct hybrid RNA precursors show that any 5' splicing site can in principle be connected to any 3' splicing site. For example, when the first exon of the early SV40 transcription unit is linked to the third exon of mouse β-globin, the hybrid intron can be spliced out to generate a perfect connection between the SV40 exon and the β -globin exon....

Lewin in the first paragraph of page 915 concludes:

> Splicing sites are **generic:** they do not have specificity for individual RNA precursors and individual precursor **do not** convey specific information (such as secondary structure) that is need for splicing.

Clearly, the position set forth by Balvay et al. regarding the role of secondary structure was mere speculation.

Even if some testing of potential intron sequences is necessary, Applicants assert it would not take undue effort to test more than one such site until a satisfactory result was achieved. As described in Example 19 of the disclosure, in a gene that was only about 2 kb there were 19 different sites that had the (C/A)AGG sequence, a result that is certainly in keeping with a two base alternative in the first position followed by a only three bases. Even the Balvay paper describes the necessary <u>exon</u> sequences as 5' (C/A)AAG- and 3'-G. In the Example that was presented, the site chosen was the most similar to the original 5' and 3' splice regions of SV40 but the other sites were also likely to be used. If one wanted to carry out a series of sites instead of a single site, a skilled practitioner could carry out the present invention systematically by proceeding from the (C/A)AGG site closest to the 5' end of the transcript since these would lead to the most truncation of the normal gene product. This would not be considered to be undue experimentation.

In view of the above arguments, Applicants assert that the rejections under 35 USC §112, first paragraph (enablement) has been overcome. Therefore, Applicants

respectfully request that the rejections be withdrawn. Applicants further assert that an enabling disclosure was provided for new claims 263-269.

Summary and Conclusions

It is Applicants belief that the pending claims are in condition for allowance. However, if a telephone conversation would further the prosecution of the present application, Applicants' undersigned attorney request that he be contacted at the number provided below.

Respectfully submitted,

Dated: 12/27/06

Cheryl H. Agris

Cheryl H. Agris, Reg. No. 34,086
Telephone No. (914) 712-0093

ENZO LIFE SCIENCES, INC.
c/o ENZO BIOCHEM, INC.
527 Madison Avenue, 9th Floor
New York, New York 10022